

and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Title:

Please replace the pending title with the following title:

G¹ Methods for Selecting Polynucleotides Encoding T Cell Epitopes

In the Substitute Specification:

Please replace the paragraph beginning on page 10, line 29 with the following paragraph:

G² FIGs. 8A and 8B. Differential expression in tumor lines of differential display clone 90. RNase protection assay: 300 picograms of clone 90 antisense probe was hybridized with 5 micrograms total RNA prior to RNase digestion and analysis of protected fragments on 5% denaturing PAGE.

Please replace the paragraph beginning on page 11, line 21 with the following paragraph:

G³ FIGs. 11A and 11B. Southern Blot Analysis of Viral Genomes p7.5/tk (FIG. 11A) and pEL/tk (FIG. 11B). The viruses v7.5/tk and vEL/tk were used to infect a well of a 6 well dish of BSC-1 cells at high multiplicity of infection (moi) and after 48 hours the cells were harvested and the DNA was isolated using DNAzol (Gibco). The final DNA product was resuspended in 50 microliters of TE 8.0 and 2.5 microliters were digested with HindIII, HindIII and ApaI, or HindIII and Not I, electrophoresed through a 1.0% agarose gel, and transferred to Nytran (Schleicher and Schuell) using a Turboblotter (Schleicher and Schuell).

The samples were probed with p7.k/tk (FIG 11A) or pEL/tk (FIG. 11B) labeled with ³²P using Random Primer DNA Labeling Kit (Bio-Rad) in QuickHyb (Stratagene). The lower portion of the figure denotes a map of the HindIII J fragment with the positions of the HindIII, NotI, and ApaI sites illustrated. The leftmost 0.5 kilobase fragment has electrophoresed off the bottom of the gel.

Please replace the paragraph beginning on page 62, line 11 with the following paragraph:

G⁴ In preliminary experiments, an average of three differentially displayed bands were identified for each pair of primers. With a total of 66 primer pairs generated from all possible combinations of 12 independent primers, approximately 200 gene fragments could be identified. In some cases multiple fragments may derive from the same gene. FIG. 7 shows the pattern of differential display fragments observed with one pair of arbitrary decamers, MR_1 (TAC AAC GAG G) (SEQ ID NO:11) and MR_5 (GGA CCA AGT C) (SEQ ID NO:13). A number of bands can be identified that are associated with all four tumors but not with the parental cells. This distribution is unrelated to the immunogenicity of the tumor cells, since only three of the four tumors are immunologically crossreactive. In contrast to the differentially expressed bands identified by RDA, which gave positive results on the Northern blots exposed for only a few hours, fragments identified by differential display did not give a signal on Northern blots even after several days. Differential expression of the differential display fragments can, however, be confirmed by RNase protection assays or by semi-quantitative PCR with sequence specific primers. An example is shown in FIGs. 8A and 8B the results of an RNase protection assay with clone 90 from differential display band 9. This

sequence, which has no significant homology to entries in the GenBank database, is expressed in all four tumor lines but not in the parental B/c.N.

Please replace the paragraph beginning on page 76, line 5 with the following paragraph:

G⁵
One well of a 6 well dish of BSC-1 was infected with v7.5/tk or vEL/tk at high multiplicity of infection (moi) and after 48 hours the cells were harvested, pelleted by low speed centrifugation, rinsed with Phosphate-Buffered Saline (PBS), and the DNA was isolated using DNazol (Gibco). The final DNA product was resuspended in 50 microliters of TE (10mM TrisHCl, pH 8.0, 1 mM EDTA) and 2.5 microliters were digested with HindIII, HindIII and ApaI, or HindIII and NotI, electrophoresed through a 1.0% agarose gel, and transferred to Nytran (Schleicher and Schuell) using a Turboblottter (Schleicher and Schuell). The samples were probed with p7.5/tk (FIG. 11A) or pEL/tk (FIG. 11B) labeled with ³²P using Random Primer DNA Labeling Kit (Bio-Rad) in QuickHyb (Stratagene) and visualized on Kodak XAR film.

Please replace the paragraph beginning on page 80, line 27 with the following paragraph:

G⁶
The genomes for vEL/tk and v7.5/tk were analyzed by Southern blotting to confirm the location of the ApaI and NotI sites in the HindIII J fragment as shown in FIGs. 11A and 11B. The filters were hybridized to ³²P labeled HindIII J fragment derived from the p7.5/tk or pEL/tk. The genomes for v7.5/tk and vEL/tk have an ApaI site that does not appear in vNotI/tk (compare lanes 7 and 8 to lane 5 in each blot) whereas digestion with NotI and HindIII yield a set of fragments of equivalent size. The 0.5 kilobase HindIII/NotI or